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Constellation of Congenital Abnormalities in an Infant: A New Syndrome or Tissue-Specific Mosaicism for Trisomy 18?

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A newborn infant born to consanguineous (first cousin) parents was noted to have complex congenital heart defect and minor anomalies suggestive of trisomy 18. Blood lymphocyte and skin fibroblast karyotypes were normal. He died in the neonatal period of postoperative complications. On interphase fluorescence in-situ hybridization (FISH) using autopsy specimens, a significant number of cells in the liver (17%) were trisomic for chromosome 18, compared to normal control liver tissue. However, interphase FISH analyses of blood lymphocytes, skin fibroblasts, and kidney tissue were normal. It is our opinion that this apparent mosaicism for trisomy 18 in the patient's liver may be spurious, though it brings into focus the issue of possible tissue/organ-specific mosaicism. The anomalies in this infant do not resemble a previously described malformation syndrome. Parental consanguinity raises the possibility that this represents a new autosomal recessive malformation syndrome.

KEY WORDS: trisomy 18, mosaicism, interphase FISH, Pena-Shokeir phenotype

INTRODUCTION

The evaluation of infants with multiple malformations poses a diagnostic challenge. Laboratory testing, including traditional cytogenetics and newer techniques such as fluorescence in-situ hybridization (FISH), may make or clarify the diagnosis in some of these cases [Drut et al., 1992]. However, the results may be ambiguous, making the interpretation difficult. This may pose a dilemma to the clinician, both in diagnosis and in counselling.

Our patient was born with complex congenital heart defect and other congenital anomalies suggestive of a chromosomal abnormality, possibly trisomy 18. Karyotypic analysis of blood lymphocytes was normal. Since the constellation of findings did not resemble other known malformation syndromes, a more rigorous search for a chromosomal anomaly was pursued. The results of interphase FISH on liver cells from the patient bring into focus the issues of tissue-specific mosaicism and its significance, the ambiguities of interphase FISH analysis, and the difficulties of counseling consanguineous parents.

MATERIALS AND METHODS

Clinical Report

Our patient, a male infant, was born at 36 weeks of gestation, to a 16-year-old Caucasian primigravida; she and husband were first cousins. The pregnancy was complicated by gestational diabetes. The infant's birth weight was 2,235 g (25th centile), length 45 cm (25th centile) and head circumference 32 cm (25th centile). Poor respiratory effort necessitated endotracheal intubation, which was difficult due to a small mandible. Several congenital anomalies were noted. The occiput was prominent. Bilateral epicanthal folds with telecanthus were present. The palpebral fissures were narrow. The base of the nose was broad and flat, with a poorly developed nasal tip and alae nasi (Fig. 1). The ears were apparently low-set and posteriorly angulated, with deficient superior helices and prominent antihelices. There were large preauricular skin tags bilaterally (4 on the left, 5 on the right), and bilateral preauricular sinuses (Fig. 2). Micrognathia and a cleft of the soft palate were evident. Overlap of the 2nd and 5th fingers over the 3rd and 4th fingers, and 5th finger clinodactyly were seen bilaterally. The fingernails and toenails were hypoplastic. On dermal ridge pattern analysis, there was an excess of arch patterns (6/10). Neurologic examination was remarkable for irritability, increased tone, and exaggerated deep tendon reflexes.

Since he had symptoms and signs of a cyanotic heart defect, an echocardiogram and cardiac catheterization were performed and the diagnoses of total anomalous

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Fig. 1. Frontal view of patient showing broad nasal root, narrow palpebral fissures, telecanthus, deficient alae nasi, apparently low-set ears and micrognathia.

pulmonary venous return, ventricular septal defect and a patent ductus arteriosus were made. Results of ultrasound examination of the kidneys and head were normal. No vertebral anomalies were seen on radiographic examination. Auditory brainstem-evoked response showed bilateral moderate hearing loss.

Surgical repair of the heart defect was performed at 2 weeks of age, with simultaneous excision of the preauricular skin tags. A gastrostomy feeding tube was placed at age 4 weeks due to feeding difficulties. Postoperatively, he developed airway complications and died. A limited autopsy was performed which confirmed the earlier findings. No other internal anomalies were found.

Fig. 2. Side view demonstrating the prominent occiput, malformed ears, preauricular tags (after excision) and the small chin.

Cytogenetic Analysis

Blood lymphocytes and skin fibroblasts were cultured, harvested and stained with GTG according to standard procedures. Karyotypes were designated according to Harnden and Klinger [1985].

Fluorescence In Situ Hybridization

Paraffin-embedded sections of 2 μ thickness from the patient's liver and kidney were obtained at autopsy. Sections of the same thickness from normal liver and kidney served as negative control tissues. Liver sections from a fetus known to have trisomy 18 by cytogenetic analysis served as the positive control. All sections were processed in our pathology department and had been handled in a standard manner. Chromosome 18 (D18Z1) and chromosome 12 (D12Z3) alpha satellite probes were obtained from Oncor (Gaithersburg, MD). The chromosome 12 alpha satellite probe served as a probe control. The protocol for hybridization was as recommended by the manufacturer. Detection and amplification was according to the method of von Kap-Herr et al. [1992].

Subsequently, GTG-banded slides of blood lymphocytes and skin fibroblasts from the patient were hybridized with D18Z1 [Smit et al., 1990] for interphase FISH analysis. Slides of blood lymphocytes and skin fibroblasts from karyotypically normal individuals hybridized with D18Z1 were used as controls.

At least 200 interphase nuclei were scored from each slide by two observers in a "blinded" fashion, according to the criteria of Hopman et al. [1988]. Only those nuclei that were discrete were scored. Damaged, overlapping, stacked nuclei, and nuclei with no signals were discounted. Coalesced and nonspecific signals were not counted. Spots in a paired arrangement were scored as one. The percentage of nuclei with 1, 2, 3, and 4 signals were calculated from the absolute numbers. The patient and the control values were compared by the chi-square test.

RESULTS

Cytogenetic Analysis

The karyotype from blood lymphocytes as well skin fibroblasts was 46,XY. Twenty cells from the blood lym-

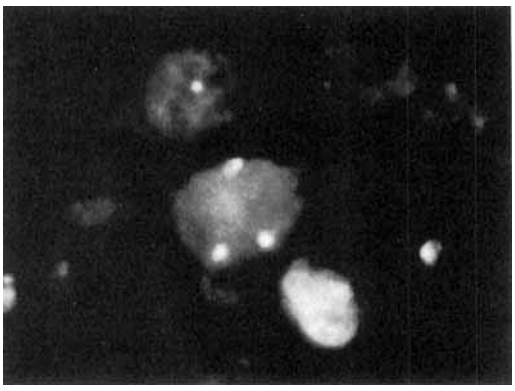

Fig. 3. Representative nuclei from the liver tissue of the patient, demonstrating 3 signals with the chromosome 18 centromeric probe.

TABLE I. Summary of FISH Results on Patient's Liver Compared to the Negative and Positive Control Tissues

			% of nuclei with signals			
Tissue	DNA probe	Nuclei counted	1	2	3	4
Normal livers	α18	600	32.6	64.0	3.3	0.1
Patient's liver	α18	400	31.15	51.6	16.95*	0.3
Trisomy 18 liver	α18	400	15.25	51.5	33.25	0

* P value <0.001 (comparison of number of nuclei with 3 signals in patient's liver tissue with mean value in normal liver tissue).

phocytes, and 100 cells from the skin fibroblasts were examined.

Fluorescence In Situ Hybridization

FISH with D18Z1 on the patient's liver cells showed 3 discrete signals in 17% of the nuclei (Fig. 3). In contrast, only 1.5–5.2% of the nuclei from the two normal control liver sections exhibited 3 signals ($P < 0.001$; Tables I and II). A larger number of nuclei (33.25%) with 3 signals was found in the liver section of the fetus with known trisomy 18, which served as a positive control (Table II). The number of cells (0.4–5%) with 3 signals for chromosome 18 in the patient's kidney, blood lymphocytes and skin fibroblasts (Table III) was not significantly different from the controls (0.4–5.2%). When the chromosome 12 alpha satellite probe was hybridized to both liver and kidney sections from the patient, 3 signals were seen in 1.3% and 3.3% of nuclei, respectively (Table III). Interobserver variability was minimal.

DISCUSSION

This male infant presented with multiple congenital abnormalities that were not completely diagnostic of a previously described malformation syndrome. A search of the POSSUM database showed trisomy 18 and Pena-Shokeir syndrome type I to be close matches. The anomalies were incompatible with those seen in an infant of a diabetic mother. The prominent occiput, narrow palpebral fissures, epicanthal folds with telecanthus, broad nasal bridge, micrognathia, overlapping fingers, increased frequency of arch patterns on the fingers, hypoplastic nails, muscle spasticity, and cardiac malformations were all suggestive of trisomy 18, although preauricular tags and sinuses are not common in trisomy 18. While nonmosaic trisomy 18 and structural chromosomal abnormalities were excluded by cytogenetic analysis of blood lymphocytes and skin fibroblasts, we opted to perform interphase FISH to exclude the possibility of an undetected low-grade mosaicism for trisomy 18. The presence of a significant number of cells (17%) with 3 signals for chromosome 18 in liver tissue of the patient raises the issue of organ/tissue-specific mosaicism for trisomy 18. There are several possible explanations for the FISH results in this case. First, this may represent true tissue/organ-specific (liver) mosaicism for trisomy 18, with loss of the trisomic cell line in the other tissues studied. In this event, the phenotype could be related to the trisomic cell line.

Tissue-specific mosaicism has been reported for trisomy 21 [Yokoyama et al., 1992], with the ratio of trisomic cells varying from one tissue to another. In other instances trisomic cells are seen in one tissue (skin fibroblasts), but absent in another (blood lymphocytes) [Hall, 1988]. The argument could be made that in our patient the trisomic cell line may have been lost in skin, lymphocytes, and kidneys during mitosis, leading to a disomic pattern. Second, the number of trisomic cells in the liver tissue (175) may be considered "normal" and not indicative of true mosaicism, although one has to consider that this result may be an underestimation of the actual number of cells with 3 signals because of nuclear truncation that occurs when paraffin sections of 2 μ thickness are cut. Up to 11% of uncultured amniocytes from karyotypically normal fetuses have been reported to exhibit 3 signals for chromosome 18 [Christensen et al., 1992]. Finally, a certain level of true chromosomal mosaicism may be a common phenomenon in normal tissues. Mosaic trisomies 7, 10 and 18 have been described in non-neoplastic kidney tissue on cytogenetic analysis [Casalone et al., 1992; Emanuel

TABLE II. FISH Results on Normal Control Tissues

			% of nuclei with signals			
Tissue	DNA probe	Nuclei counted	1	2	3	4
Liver (control 1)	α18	200	29.45	68.9	1.5	0.15
Liver (control 2)	α18	400	35.8	59.0	5.2	0
Trisomy 18 liver	α18	400	15.25	51.5	33.25	0
Kidney	α18	500	33.8	61.0	5.2	0
Fibroblasts	α18	500	0.2	98.4	1.4	0
Blood lymph	α18	500	0.2	99.4	0.4	0

TABLE III. Details of FISH Results on Patient's Tissues

Tissue	DNA probe	Nuclei counted	% of nuclei with signals			
			1	2	3	4
Liver	α18	400	31.15	51.6	16.95	0.3
	α12	300	49.7	49.0	1.3	0
Kidney	α18	1,000	38.0	57.0	5.0	0
	α12	600	42.4	54.3	3.3	0
Fibroblasts	α18	500	0.4	99.2	0.4	0
Blood lymphocytes	α18	1,000	3.2	94.8	1.8	0.2

et al., 1992] as well as by FISH (2–18% of cells) [Emanuel et al., 1992]. It is postulated that these trisomic cells may arise due to nondisjunction in rapidly dividing embryonal cells [Vogel and Motulsky, 1986]. When this nondisjunction occurs late in organ development, only a few cells are affected, leading to mosaicism. Some families apparently have chromosomes that are predisposed to anaphase lag or nondisjunction during mitosis, leading to somatic mosaicism [Juberg et al., 1988].

It is unlikely that our FISH findings are artifactual, since strict scoring criteria were applied and the interobserver variability was minimal. Furthermore, the use of appropriate negative and positive tissue control sections validates our results [Anastasi et al., 1990; Shashi et al., 1994]. The phenomenon of tissue-specific mosaicism may be more common than previously thought. As more cases are studied, other instances may be found where blood lymphocytes and skin fibroblasts are normal and other tissues are not. Interphase cytogenetic analysis on fetal and placental tissues may be of value in further understanding tissue-specific mosaicism. We were not able to study other tissues in our patient due to lack of parental consent.

An alternative diagnosis was the Pena-Shokeir syndrome type I. Although our patient had micrognathia, epicanthal folds, ear abnormalities, overlapping fingers, abnormal dermatoglyphics, and congenital heart disease, which occur in this condition, other diagnostic features such as intrauterine growth retardation, contractures, pulmonary hypoplasia and polyhydramnios were absent [Hall, 1986]. Preauricular skin tags and sinuses are not manifestations of Pena-Shokeir phenotype.

We considered if our patient could have the oculoauricular-vertebral (OAV) spectrum. Although ear malformation, preauricular tags and sinuses, hearing loss, mandibular hypoplasia, cleft palate and heart defect were compatible with this diagnosis, ocular anomalies and vertebral anomalies were absent as was facial asymmetry. In addition, our patient's facial anomalies, overlapping fingers and dermatoglyphics were not suggestive of the OAV spectrum.

In the absence of a chromosomal abnormality and lack of concordance with a known malformation syndrome, the multiple anomalies in this infant may represent a hitherto undescribed malformation syndrome. Parental consanguinity strengthens the speculation that it could be inherited as an autosomal recessive trait. More case reports are needed to further delineate this entity.

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